

# Elevated plasma concentration of reduced homocysteine in patients with human immunodeficiency virus infection<sup>1-3</sup>

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**ABSTRACT** Oxidative stress has been suggested to be an important factor in the immunopathogenesis of human immunodeficiency virus (HIV) infection. Reduced plasma thiols may lead to production of reactive oxygen species, thus contributing to the oxidative stress. We quantified the total, reduced, and protein-bound forms of the thiols homocysteine, cysteine, cysteinylglycine, and methionine in plasma from 21 HIV-infected patients and 15 healthy control subjects and compared the results with clinical and immunologic indexes. The HIV-infected patients had significantly higher concentrations of reduced homocysteine in plasma compared with control subjects. No significant differences in reduced homocysteine concentrations were noted when asymptomatic and symptomatic HIV-infected patients were compared, and we did not find any relation between reduced homocysteine concentrations and other markers of immunodeficiency. The HIV-infected patients had normal total homocysteine concentrations. The reduced cysteinylglycine concentration tended to be elevated in the patient group. No differences between HIV-infected patients and control subjects were found for reduced or total cysteine. Compared with control subjects, the HIV-infected patients had lower concentrations of methionine in plasma, and a significant correlation was found between low concentrations of methionine and low CD4<sup>+</sup> lymphocyte counts in blood. Elevated concentrations of reduced homocysteine could possibly contribute to formation of reactive oxygen species, leading to accelerated immunologic deterioration and increased HIV replication. *Am J Clin Nutr* 1996;63:242-8.

**KEY WORDS** Homocysteine, cysteine, methionine, human immunodeficiency virus, oxidative stress

## INTRODUCTION

Formation of highly reactive oxygen species (ROS) is a consequence of a variety of essential biochemical reactions. The continuous production of prooxidants is normally balanced by their consumption by antioxidants (1). Oxidative stress results from imbalance between these processes resulting in accumulation of prooxidants.

Disturbances in redox status leading to increased oxidative stress may play an important pathophysiologic role in patients infected with the human immunodeficiency virus (HIV) (2-5). Experimental data suggest that oxidative stress may impair various immune functions, such as lymphocyte activation by mitogens (6), natural killer cell activation (7), and lymphocyte-mediated cytotoxicity (8). Also, recent evidence indicates that

oxidative stress may enhance apoptosis of immunocompetent cells (9, 10). These effects of oxidative stress are all relevant for the immunopathogenesis of HIV infection. Furthermore, *in vitro* studies suggest that ROS may activate nuclear factor  $\kappa$ B (NF- $\kappa$ B) in HIV-infected cells, and thereby enhance HIV replication (11, 12).

Glutathione is the most abundant intracellular sulfhydryl compound and plays a key role in cellular detoxification, including intracellular trapping of ROS (13). Patients with HIV infection have disturbed glutathione homeostasis in many types of cells, including lymphocytes (14-16), and there are reports of low concentrations of glutathione and cysteine in plasma (17-20). In a recent report, we found that CD4<sup>+</sup> lymphocytes from HIV-infected patients were characterized by a substantial increase in oxidized glutathione concentrations and a considerable decrease in the ratio of reduced to total glutathione (21). In plasma, HIV-infected patients had significantly lower concentrations of oxidized glutathione and the concentrations of total glutathione tended to be lower compared with control subjects.

Depending on their concentration and chemical environment, plasma thiols may have a protective function, serving as antioxidants, or they may contribute to the oxidative stress by the production of ROS (22). Superoxide anion, hydrogen peroxide, and hydroxyl radical have all been detected during the oxidation of thiols (23).

Of the plasma thiols, homocysteine has recently received considerable attention, especially because it is a risk factor for early-onset cardiovascular disease (24), and is a useful marker of impaired function of cobalamin or folate (25). Notably, ROS formation has been demonstrated during autooxidation of ho-

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homocysteine (26, 27) and it has been suggested that reduced homocysteine is a possible atherogenic agent because of its prooxidant effect (22, 28).

Homocysteine is a product of transmethylation, and is either remethylated to methionine or converted to cystathionine. The former reaction is catalyzed in most tissues by cobalamin-dependent methionine synthase, which requires 5-methyltetrahydrofolate as cosubstrate (25). Cystathionine is metabolized to cysteine, which is a precursor of glutathione (29, 30). Glutathione in turn is degraded to cysteinylglycine, which is further cleaved to cysteine and glycine (29). Thus, these thiols are metabolically related.

We recently developed a procedure for the determination of reduced, oxidized, and total concentrations of homocysteine, cysteine, and cysteinylglycine in plasma. With this method we analyzed plasma from HIV-infected patients and matched control subjects. In addition, we evaluated the association between thiol concentrations in plasma and important clinical and immunologic indexes in the HIV-infected patients.

## SUBJECTS AND METHODS

### Subjects

Blood samples from 21 HIV-infected patients were obtained. Patient characteristics are summarized in **Table 1**. All patients had serum antibodies to HIV in two separate serum samples as determined by enzyme immunoassay (Organon Teknika, Bostel, Netherlands) and confirmed by Western blot (DuPont, Wilmington, DE). According to the revised criteria of the Centers for Disease Control and Prevention (CDC) (31), 9 patients were classified as asymptomatic (CDC group A) and 12 patients as symptomatic (8 in CDC group B and 4 in CDC group C).

Because of possible changes in thiol status due to causes other than HIV infection, the following categories of patients were excluded: patients with ongoing acute infection at the time of blood collection (from 3 wk before to 1 wk after), intravenous drug users or patients abusing alcohol, patients in whom antiretroviral therapy had been initiated during the previous 6 mo, and patients with serum concentrations of alanine

aminotransferase  $> 0.83 \mu\text{kat/L}$  ( $> 50 \text{ U/L}$ ) or creatinine  $> 100 \mu\text{mol/L}$ .

Eleven patients received zidovudine (median dosage: 600 mg/d; range: 300–900 mg/d) and 10 patients received anti-*Pneumocystis carinii* prophylaxis (300 mg aerosolized pentamidine every 4 wk in three patients, and 100 mg dapsone every second day in seven patients) at the time of the study. The serum albumin concentration was  $> 40 \text{ g/L}$  in all patients. Because some of the studied indexes may be influenced by malabsorption, presence of weight loss or diarrhea was recorded. There was no weight loss, defined as a loss of body weight  $> 10\%$  during 1 mo, in any of the patients before the study. None of the patients suffered from diarrhea, defined as more than two loose stools per day for at least 1 mo.

Control subjects were 15 sex- and age-matched healthy, unpaid, HIV-seronegative (by enzyme immunoassay) blood donors (Table 1). All patients and control subjects were without family history of coronary heart disease. The percentage of smokers was equal in the patient and control groups (Table 1). Informed consent was obtained from all participants.

### Blood-sampling protocol

Blood samples were drawn between 0800 and 1000 after an overnight fast. For serum sampling, sterile vacuum tubes of blood without additives were immediately immersed in melting ice and the blood was allowed to clot for  $< 1 \text{ h}$  before centrifugation at  $400 \times g$  for 10 min. Sera were stored at  $-80^\circ\text{C}$  until analyzed. For determination of various thiol components in plasma, blood was routinely collected into three evacuated tubes and placed in melting ice containing heparin as anticoagulant with isotonic solutions containing either monobromobimane (mBrB; Molecular Probes, Eugene, OR) or *N*-ethylmaleimide (NEM; Sigma Chemical Co, St Louis) as thiol-derivatizing reagents or no additive (32). The blood was centrifuged within 15 min ( $400 \times g$ ,  $4^\circ\text{C}$ , 10 min). Plasma was then immediately centrifuged at  $10\,000 \times g$  at  $4^\circ\text{C}$  for 5 min to obtain platelet-free plasma.

### Determination of reduced, free oxidized, and total homocysteine, cysteine, and cysteinylglycine in plasma

Assays of reduced, free oxidized, and total forms of homocysteine, cysteine, and cysteinylglycine were described in detail elsewhere (32). The amounts of reduced thiols were obtained from the mBrB-treated plasma samples. Reduced thiols react with mBrB and form fluorescent adducts, which were quantified by reversed-phase ion-pair liquid chromatography and fluorescence detection. Free oxidized forms were quantified in NEM-treated plasma. NEM blocks free sulfhydryl groups enabling the selective determination of oxidized forms. Samples were treated with sulfosalicylic acid, and the disulfides reduced by sodium borohydride ( $\text{NaBH}_4$ ; Fluka Chemie AG, Switzerland) and then derivatized with mBrB and quantified as above.

Total amounts of homocysteine, cysteine, and cysteinylglycine were obtained from untreated plasma. Free and protein-bound disulfides in the samples were reduced by  $\text{NaBH}_4$  and thiols were derivatized with mBrB; the thiol-bimane adducts were quantified as described above. The protein-bound fraction was determined by subtracting the reduced and free oxidized species from the total amount. For each of the thiols, the ratio

**TABLE 1**  
Clinical and immunologic characteristics of the study group<sup>1</sup>

	HIV patients <sup>2</sup> (n = 17M, 4F)	Control subjects (n = 12M, 3F)
Age (y) <sup>3</sup>	36 (21–60)	35 (28–53)
Smokers		
(n)	10	7
(%)	48	46
CD4 <sup>+</sup> lymphocytes ( $\times 10^6/\text{L}$ )	110 <sup>d</sup> (50–245)	550 (340–750)
CD8 <sup>+</sup> lymphocytes ( $\times 10^6/\text{L}$ )	590 <sup>d</sup> (420–790)	315 (260–350)
Neopterin (nmol/L)	19.8 (14.4–25.8)	ND
Serum tumor necrosis factor $\alpha$ (ng/L)	29 <sup>d</sup> (20–52)	8 (0–10)

<sup>1</sup> Medians; 25th–75th percentiles in parentheses. ND, not determined.

<sup>2</sup> Nine patients were classified as asymptomatic [CDC (Centers for Disease Control and Prevention) group A] and 12 as symptomatic (8 in CDC group B and 4 in CDC group C).

<sup>3</sup> Medians and ranges in parentheses.

<sup>4</sup> Significantly different from control subjects,  $P < 0.001$ .

of reduced to total species was determined as a measure of the redox status of that thiol. Processed and derivatized samples were stored at  $-80^{\circ}\text{C}$  (median storage time: 8 wk; range: 4–16 wk) until chromatographic analysis and were frozen and thawed only once. The various thiol species were routinely measured in duplicate.

### Determination of methionine and lymphocyte subsets

Methionine was determined in deproteinized serum by an assay based on derivatization with *o*-phthalaldehyde and fluorescence detection (33). The numbers of  $\text{CD4}^{+}$  and  $\text{CD8}^{+}$  lymphocytes were determined by immunomagnetic quantification, which has been shown to agree well with results of flow cytometry (34).

### Interleukin 2 production in lymphocyte culture

Lymphocyte function was assessed as interleukin 2 (IL-2) production in stimulated lymphocytes. Peripheral blood mononuclear cells (PBMCs) were prepared from heparinized blood by Isopaque-Ficoll (Lymphoprep; Nycomed Pharma AS, Oslo) gradient centrifugation ( $400 \times g$ , 15 min,  $20^{\circ}\text{C}$ ) within 1 h after blood sampling. Mononuclear cells were washed twice in Hanks balanced salt solution (HBSS; Gibco, Paisley, United Kingdom) and finally resuspended at a concentration of  $10^9$  cells/L in RPMI 1640 with 2 mmol L-glutamine/L and 25 mmol HEPES buffer/L (Gibco) supplemented with gentamicin (40 mg/L) and 10% heat-inactivated pooled human AB+ serum (culture medium). The endotoxin content of the culture medium was  $< 10$  ng/L (Limulus amoebocyte lysate test, quantitative chromogenic LAL-1000; Biowhittaker, Walkersville, MD). The cells were cultured in triplicate for 7 d in 96-well microtiter trays (Costar, Cambridge, MA).

To block IL-2 consumption, 5 mg anti-CD25 antibody/L (anti-IL-2 receptor, clone 3G10; Boehringer Mannheim Biochemica, Mannheim, Germany) was added to all wells (35). Cells were stimulated with 100  $\mu\text{g/L}$  staphylococcal enterotoxin B (SEB, Sigma) or influenza virus A/Singapore/6/86 (INF; final concentration 1:1000). Patients' cells were always cultured in the same microtiter trays as control blood donor cells. Supernates were stored at  $-80^{\circ}\text{C}$  until IL-2 was determined by enzyme immunoassay (Cayman Chemical, Ann Arbor, MI).

### Quantification of neopterin, tumor necrosis factor $\alpha$ , cobalamin, and folate

The macrophage activation marker neopterin was quantified in serum by a radioimmunoassay (IMMUtest Neopterin; Henning Berlin GMBH, Berlin) according to the manufacturer's instructions. Tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) in serum, a marker of inflammatory stress, was quantified by enzyme immunoassay (Medgenix, Fleurus, Belgium) as previously described (36). Serum cobalamin and folate in serum and erythrocytes were quantified by standard methods using radioimmunoassays (Diagnostic Product Corp, Los Angeles). The normal ranges at our hospital are 120–580 pmol/L (serum cobalamin), 6.0–20.0 nmol/L (serum folate), and 450–1300 nmol/L (erythrocyte folate).

### Statistical analysis

The Mann-Whitney *U* test was used for comparison of the two groups of individuals. When more than two groups were compared, the Kruskal-Wallis test was used. If a significant difference was found, Fisher's least-significant-difference test was computed on the ranks to determine differences between each pair of groups. Coefficients of correlation (*r*) were calculated with the Spearman rank test. Data are given as medians and 25th–75th percentiles if not otherwise stated. *P* values are two-sided and were considered significant when  $< 0.05$ . The statistical software program STATISTICA (StatSoft, Tulsa, OK) was used for the analyses.

## RESULTS

### Plasma thiol concentrations

The plasma concentration of total homocysteine was similar in patients and control subjects (Table 2). Notably, the concentration of the reduced form was significantly higher in patients with HIV infection than in control subjects; only one of the 21 patients had a plasma concentration of reduced homocysteine within the range of the control subjects. Also, the patients had a significantly higher ratio of reduced to total thiol species (approximately a fourfold increase) for homocysteine compared with the control subjects as shown in Figure 1. No differences in the plasma concentrations of the free oxidized or

TABLE 2

Total, oxidized, reduced, and protein-bound thiol species in plasma from patients with human immunodeficiency virus (HIV) infection ( $n = 21$ ) and HIV-seronegative control subjects ( $n = 15$ )<sup>1</sup>

	Total	Oxidized	Reduced	Protein-bound
	$\mu\text{mol/L}$			
Homocysteine				
Control subjects	9.21 (7.51–10.22)	1.65 (1.49–2.30)	0.15 (0.09–0.24)	6.94 (5.76–8.39)
HIV-infected patients	8.97 (6.79–11.20)	1.76 (1.54–2.23)	0.49 <sup>2</sup> (0.39–0.80)	6.17 (4.50–7.92)
Cysteine				
Control subjects	318.1 (289.2–337.2)	124.9 (115.6–149.5)	10.9 (8.3–11.6)	186.7 (138.0–197.8)
HIV-infected patients	292.8 (266.5–316.5)	114.4 <sup>3</sup> (99.9–128.9)	11.1 (8.3–13.3)	159.1 (143.8–204.78)
Cysteinylglycine				
Control subjects	27.84 (23.31–36.37)	8.77 (8.28–11.84)	2.34 (1.99–2.89)	15.55 (12.01–21.53)
HIV-infected patients	23.24 <sup>3</sup> (20.59–27.4)	9.08 (6.82–10.50)	2.68 (1.80–3.11)	12.1 (9.34–16.75)

<sup>1</sup> Medians; 25th–75th percentiles in parentheses.

<sup>2,3</sup> Significantly different from control subjects: <sup>2</sup>  $P < 0.001$ , <sup>3</sup>  $P < 0.05$ .

protein-bound forms were found between patients and control subjects.

The plasma concentrations of the various cysteine species were described elsewhere (21) and are summarized in Table 2. Both the total, protein-bound, and reduced cysteine concentrations and the ratios of reduced to total cysteine were similar in patients and control subjects (Table 2). The plasma concentration of oxidized cysteine was significantly lower in the patient group, although the difference was modest (a decrease of  $\approx 10\%$ ; Table 2).

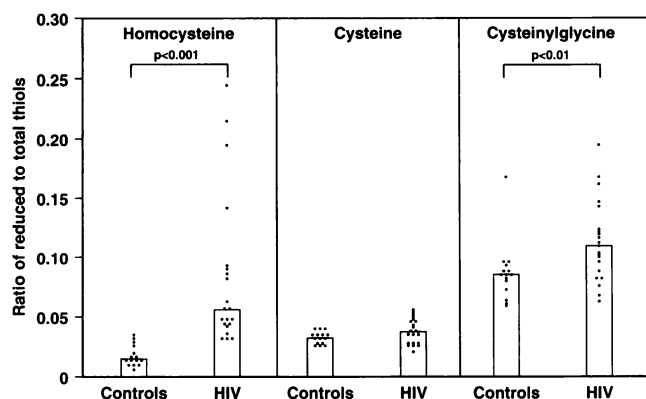
The concentration of total cysteinylglycine in plasma was significantly lower in HIV patients than in control subjects (a decrease of  $\approx 17\%$ ; Table 2), whereas there were no differences when the oxidized, reduced, or protein-bound forms were compared (Table 2). However, a significantly higher ratio of reduced to total thiol species for cysteinylglycine was found in the HIV patients (an increase of  $\approx 30\%$ ; Figure 1).

The concentration of methionine in plasma was  $49.8 \mu\text{mol/L}$  (range:  $44.8\text{--}58.2 \mu\text{mol/L}$ ) in the control subjects compared with  $45.1 \mu\text{mol/L}$  (range:  $38.4\text{--}47.9 \mu\text{mol/L}$ ) in patients with HIV infection ( $P < 0.01$ ). The methionine concentration in the asymptomatic patients,  $48.5 \mu\text{mol/L}$  (range:  $42.7\text{--}49.2 \mu\text{mol/L}$ ), was similar to that of the control subjects. Thus, the reduction in plasma methionine was confined to the symptomatic patients:  $41.1 \mu\text{mol/L}$  (range:  $38.3\text{--}46.4 \mu\text{mol/L}$ ;  $P < 0.01$  compared with control subjects,  $P < 0.02$  compared with asymptomatic patients).

No significant correlation was found between methionine and total homocysteine ( $r = -0.25$ ) in the patient group. A significant correlation was found between methionine and the number of  $\text{CD4}^+$  lymphocytes in blood ( $r = 0.55$ ;  $P = 0.01$ ) whereas no significant correlations were found between methionine and neopterin,  $\text{TNF-}\alpha$ , IL-2 production in lymphocyte culture, or the number of  $\text{CD8}^+$  lymphocytes in blood in the HIV-infected patients (data not shown).

#### Blood concentrations of cobalamin and folate

The median serum concentration of cobalamin in the HIV-infected patients was  $250 \text{ pmol/L}$  (range:  $155\text{--}290 \text{ pmol/L}$ ). Nineteen of the patients had cobalamin concentrations within



**FIGURE 1.** Ratios between reduced and total plasma concentrations of the thiols homocysteine, cysteine, and cysteinylglycine in 15 healthy control subjects and 21 HIV-infected patients. Bars represent medians. Significant differences by the Mann-Whitney  $U$  test (two-sided) are indicated.

the normal range, whereas the concentration was 75 and 115  $\mu\text{mol/L}$  in the remaining two patients. These two patients had moderately elevated total homocysteine concentrations of 13.4 and 14.2  $\mu\text{mol/L}$ .

The concentration of erythrocyte folate was within the normal range in 17 of the HIV-infected patients, whereas the remaining 4 had slightly reduced concentrations: 375, 395, 405, and 415  $\text{nmol/L}$ . The plasma concentrations of the various homocysteine species in these four patients were similar to those of the other patients (data not shown). All patients had normal concentrations of serum folate. There were no significant correlations between plasma total homocysteine and the concentrations of serum cobalamin, erythrocyte folate, or serum folate.

#### Redox status of thiol species and immunologic indexes

When the ratios of reduced to total plasma thiols were compared, significant correlations between cysteine and cysteinylglycine were found both in the patients ( $r = 0.78$ ,  $P < 0.01$ ) and in the control subjects ( $r = 0.65$ ,  $P = 0.01$ ). No significant correlations were found between ratios of homocysteine to cysteinylglycine or between ratios of homocysteine to cysteine in the patients or in the control group.

When plasma concentrations of the various thiol species in patients with asymptomatic and symptomatic HIV infection were compared, no significant differences were found (data not shown). No significant correlations were found when the number of  $\text{CD4}^+$  lymphocytes or  $\text{CD8}^+$  lymphocytes in blood,  $\text{TNF-}\alpha$  in serum, neopterin in serum, or lymphocyte IL-2 production in response to SEB or INF were compared with the various forms of homocysteine, cysteine, or cysteinylglycine in plasma in the patients (data not shown).

#### DISCUSSION

A major finding of this study is that all but one of the patients with HIV infection had plasma concentrations of reduced homocysteine above the range of the control group. No difference in the plasma concentration of total homocysteine was found when patients and control subjects were compared. As a result, the ratio of reduced to total homocysteine was considerably increased in the HIV-infected patients. Our data on plasma concentrations of total homocysteine in HIV-infected patients agree with those of a previous study showing normal serum concentrations of total homocysteine in 20 patients with acquired immunodeficiency syndrome (AIDS) (18). However, plasma concentrations of reduced homocysteine in HIV-infected patients were not determined in previous studies.

Elevated concentrations of reduced homocysteine in plasma have been found in persons with various other conditions such as homocystinuria (37) and cobalamin deficiency (38), and after administration of homocysteine (39) or methionine (40). In all these instances, the elevation of reduced homocysteine was associated with increased concentrations of total homocysteine in plasma. To our knowledge, the combination of elevated concentrations of reduced homocysteine and normal total homocysteine concentrations has not been observed in any clinical condition.

Depending on indexes such as pH, concentration, and the presence of metal ions, reduced homocysteine may function

either as an antioxidant or as a prooxidant (22, 28). In patients with an elevated plasma concentration of reduced homocysteine and early-onset arteriosclerosis, reduced homocysteine has been suggested as a possible atherogenic agent because of its prooxidant effect (28).

Our finding of elevated concentrations of reduced homocysteine may be relevant for current theories implicating oxidative stress in the immunopathogenesis of HIV infection (2, 4, 5, 21). Elevated concentrations of reduced homocysteine might contribute to the production of ROS because the sulfhydryl group of homocysteine is believed to act catalytically with cupric or ferric ions to generate hydrogen peroxide and various homocysteinyl radicals (23, 26, 27). In one of these studies, homocysteine plus an increasing concentration of copper led to hydrogen peroxide production in a dose-dependent manner (27). Although data on serum copper concentrations in HIV-infected patients are discrepant (41, 42), a longitudinal study has shown that patients progressing to AIDS had significantly higher serum copper concentrations compared with the non-progressors (41). In the presence of metal ions (eg, copper or iron ions), hydrogen peroxide can react to form the highly reactive hydroxyl radical (23). Recent studies suggest that it is the hydroxyl radical that is responsible for the NF- $\kappa$ B activation causing the stimulation of HIV replication in HIV-infected cells (43). Thus, one may speculate that elevated circulating concentrations of reduced homocysteine in HIV-infected patients could be one of several factors contributing to an enhanced production of ROS, which in turn could lead to stimulation of HIV replication through NF- $\kappa$ B activation. Alternative to the hypothesis suggested above, elevated concentrations of reduced homocysteine might be a consequence of other redox disturbances in patients with HIV infection. Also, it is uncertain whether the measured reduced homocysteine in plasma from these patients is trapped in a form reacting with the derivatizing agent or exists as authentic homocysteine in the circulation.

No significant differences in reduced homocysteine concentrations were noted when asymptomatic and symptomatic HIV-infected patients were compared, and we did not find any relation between reduced homocysteine concentrations and other markers of immunodeficiency.

It is known that factors such as food intake (44), age (45), sex (45), smoking (46), and a family history of coronary heart disease (30) may all be related to the homocysteine concentration in plasma. All these factors were controlled for in the present study. Also, cobalamin or folate deficiency leads to hyperhomocysteinemia (30, 38). Most patients in our study had cobalamin concentrations in the lower normal range. Although only two of the patients had serum cobalamin concentrations below normal, their plasma concentrations of total homocysteine were slightly elevated. Our finding of subnormal cobalamin concentrations in some HIV-infected patients agrees with that of recent studies (42, 47, 48). However, the total homocysteine concentrations in the patient and control groups were similar, and there were no correlations between serum cobalamin or erythrocyte folate and total homocysteine.

Our finding of a reduced methionine concentration in plasma from HIV-infected patients agrees with the findings of previous reports (17, 20). Because methionine is an essential amino acid, malabsorption may lead to methionine deficiency. However,

none of the patients in this study had clinical signs or symptoms suggesting malabsorption.

A significant but modest decrease in the total cysteinylglycine plasma concentration was found in the HIV-infected patients compared with control subjects. Because cysteinylglycine is a degradation product of glutathione (49), the decreased concentration of cysteinylglycine might reflect a low concentration of glutathione. In fact, plasma glutathione tended to be lower in this group of HIV-infected patients than in control subjects (21). The reduced cysteinylglycine concentration tended to be slightly elevated in the patient group, resulting in an increased ratio of reduced to total thiol species. As with homocysteine, elevated concentrations of reduced cysteinylglycine might contribute to ROS generation because of the presence of a free sulfhydryl group (23).

As discussed elsewhere (21), we found normal plasma concentrations of the various forms of cysteine, apart from a slight decrease in oxidized cysteine in plasma from HIV-infected patients. Our data agree with those of a previous report showing no significant differences in serum cysteine concentrations between AIDS patients and control subjects (18). Previously, several investigators found decreased plasma concentrations of cysteine in HIV-infected patients (17, 19, 20, 50).

Determination of thiol status in plasma is particularly demanding because redox conditions change within seconds after blood collection (23, 29, 32). Reduced thiols might also be decreased *ex vivo* as a result of the formation of protein-bound disulfides (51), leading to underestimation of the free thiol fraction if necessary steps are not taken. The kinetics of this *ex vivo* formation of protein-bound disulfides might be different in the patient and control groups, causing further difficulties in the interpretation of thiol concentrations. These problems are avoided in the present study due to the immediate thiol derivatization during blood collection (32).

In the patients with HIV infection, we found a significant correlation between the ratios of reduced to total cysteine and cysteinylglycine, suggesting disulfide interchange reactions leading to an equilibrium state as previously described (38, 39). In contrast with previous studies (38, 39), no such correlations were found between the ratio of reduced to total homocysteine or between corresponding ratios of the other thiols, neither in the HIV-infected patients nor in the control subjects.

Treatment of HIV-infected patients with antioxidants such as *N*-acetylcysteine to reduce oxidative stress has been suggested by several researchers (4, 52). Little is known about the effect of antioxidants on plasma concentrations of reduced homocysteine, thus it is important that homocysteine quantifications should be included when HIV-infected patients are monitored during clinical trials with antioxidants.

In conclusion, patients with HIV infection have elevated plasma concentrations of reduced homocysteine. High concentrations of reduced homocysteine could possibly be one of several contributing factors to the formation of reactive oxygen species causing NF- $\kappa$ B activation, enhanced HIV replication, and accelerated immunologic deterioration. ■

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